

WHAT IS CLAIMED IS:

1. A composition comprising an isolated DNA molecule comprising one or more copies of TRT (SEQ ID NO:3), or a functional variant thereof, with the proviso that the DNA molecule does not comprise the entire sequence of TRT" (SEQ ID NO:4).

2. The composition of claim 1 wherein the DNA molecule comprises one or more copies of TRT (SEQ ID NO:3), or a functional variant thereof, and a heterologous nucleotide sequence.

3. The composition of claim 1 wherein the DNA molecule comprises one or more copies of TRT (SEQ ID NO:3), or a functional variant thereof, with the proviso that the DNA molecule does not comprise more than 200 contiguous nucleotides of the sequence of TRT" (SEQ ID NO:4).

4. The composition of claim 3 wherein the DNA molecule does not comprise more than 100 contiguous nucleotides of TRT" (SEQ ID NO:4).

5. The composition of claim 3 wherein the DNA molecule does not comprise more than 32 contiguous nucleotides of the sequence of TRT" (SEQ ID NO:4).

6. A composition comprising an isolated DNA molecule comprising one or more copies of TRT' (SEQ ID NO:2), or a functional variant thereof, with the proviso that the DNA molecule does not comprise the entire sequence of TRT" (SEQ ID NO:1).

7. The composition of claim 6 wherein the DNA molecule comprises one or more copies of TRT' (SEQ ID NO:2), or a functional variant thereof, and a heterologous nucleotide sequence.

8. The composition of claim 6 wherein the DNA molecule comprises one or more copies of TRT' (SEQ ID NO:2), or a functional variant thereof, with the proviso that the DNA molecule does not comprise more than 200 contiguous nucleotides of the sequence of TRT" (SEQ ID NO:4).

9. The composition of claim 8 wherein the DNA molecule does not comprise more than 150 contiguous nucleotides of TRT" (SEQ ID NO:4).

10. The composition of claim 8 wherein the DNA molecule does not comprise more than 116 contiguous nucleotides of TRT" (SEQ ID NO:4).

11. The composition of any one of claims 1 to 10 wherein the DNA molecule
5 further comprises a selectable marker.

12. The composition of any one of claims 1 to 10 wherein the DNA molecule is a vector.

10 13. A cell transformed with a DNA molecule, said DNA molecule comprising one or more copies of TRT (SEQ ID NO:3), or a functional variant thereof, with the proviso that the DNA molecule does not comprise the entire sequence of TRT" (SEQ ID NO:4).

14. A cell transformed with a DNA molecule, said DNA molecule comprising one
15 or more copies of TRT' (SEQ ID NO:2), or a functional variant thereof, with the proviso that the DNA molecule does not comprise the entire sequence of TRT" (SEQ ID NO:4).

15. The cell of claim 13 or 14 wherein the DNA molecule is integrated into the chromosome of the cell.

20 16. A eukaryotic cell transformed with a DNA molecule integrated into its chromosome, said DNA molecule comprising one or more copies of TRT (SEQ ID NO:3) or a functional variant thereof, or TRT' (SEQ ID NO:2), or a functional variant thereof.

25 17. The cell of claim 16 which is a mouse embryonic stem cell.

18. The cell of claim 16 wherein the DNA molecule comprises two copies of TRT' (SEQ ID NO:3), or a functional variant thereof, separated by a heterologous nucleotide sequence.

30 19. The cell of claim 16 wherein the DNA molecule comprises two copies of TRT' (SEQ ID NO:2), or a functional variant thereof, separated by a heterologous nucleotide sequence.

35 20. A kit comprising in one or more containers:
a) an isolated DNA molecule comprising one or more copies of TRT (SEQ ID NO:3) or a functional variant thereof; and

- b) an isolated TnpI protein, a TnpI expression vector or a cell capable of expressing TnpI.
21. A kit comprising in one or more containers:
 - a) an isolated DNA molecule comprising one or more copies of TRT' (SEQ ID NO:2) or a functional variant thereof; and
 - b) an isolated TnpI protein, a TnpI expression vector or a cell capable of expressing TnpI.
22. A method for effecting TnpI-mediated site-specific recombination comprising exposing a first TnpI recombination target site and a second TnpI recombination target site with TnpI protein, under sufficient conditions and in an amount sufficient to mediate site-specific recombination between the first and second TnpI recombination target sites, wherein the first and second TnpI recombination target sites are selected from the group consisting of TRT (SEQ ID NO:3) or a functional variant thereof, and TRT' (SEQ ID NO:2) or a functional variant thereof, with the proviso that the DNA molecule does not comprise the entire sequence of TRT" (SEQ ID NO:4).
23. The method of claim 22 wherein the site-specific recombination occurs *in vitro*.
24. The method of claim 22 wherein the site-specific recombination occurs in a cell.
25. The method of claim 24 wherein the cell is a non-*Bacillus thuringiensis* cell.
26. The method of claim 24 wherein the cell is not a Gram positive cell.
27. The method of claim 22 wherein the first TnpI recombination target site and the second TnpI recombination target site are on different DNA molecules.
28. The method of claim 22 wherein the first TnpI recombination target site and the second TnpI recombination target site are on the same DNA molecule.
29. The method of claim 28 wherein the DNA molecule is chromosomal DNA.

30. The method of claim 28 wherein the first TnpI recombination target site and the second TnpI recombination target site are in direct orientation.

31. The method of claim 28 wherein the DNA molecule further comprises one or
5 more non-TnpI site-specific recombination target sites.

32. The method of claim 31 wherein at least one of the one or more non-TnpI sites is a Cre recombinase target site.

10 33. The method of claim 31 wherein at least one of the one or more non-TnpI sites is a Flp recombinase target site.

34. The method of claim 31 wherein the DNA molecule comprises, in the following order, from 5' to 3', the first TnpI recombination target site, a heterologous
15 nucleotide sequence, and the second TnpI recombination target site.

35. A method for effecting site-specific recombination in a non-*Bacillus thuringiensis* cell comprising exposing a first TnpI recombination target site and a second TnpI recombination target site to TnpI protein, under sufficient conditions and in an amount
20 sufficient to mediate site-specific recombination between the first TnpI recombination target site and second TnpI recombination target site, wherein the first and second TnpI recombination target sites are selected from the group consisting of TRT (SEQ ID NO:3) or a functional variant thereof, and TRT' (SEQ ID NO:2) or a functional variant thereof.

25 36. The method of claim 35, further comprising before said exposing step, a step of introducing into the cell a first TnpI recombination target site and a second TnpI recombination target site.

37. The method of claim 35 wherein the first TnpI recombination target site and
30 the second TnpI recombination target site are on different DNA molecules.

38. The method of claim 35 wherein the first TnpI recombination target site and the second TnpI recombination target site are on the same DNA molecule.

35 39. The method of claim 38 wherein the first TnpI recombination target site and
the second TnpI recombination target site are in direct orientation.

40. The method of claim 38 wherein the DNA molecule further comprises one or more non-TnpI site-specific recombination target sites.

41. The method of claim 40 wherein at least one of the one or more non-TnpI sites is a Cre recombinase target site.

42. The method of claim 40 wherein at least one of the one or more non-TnpI sites is a Flp recombinase target site.

43. The method of claim 38 wherein the DNA molecule further comprises a heterologous nucleotide sequence, in the following order, from 5' to 3', the first TnpI recombination target site, a heterologous nucleotide sequence, and the second TnpI recombination target site, such that recombination between the first and the second TnpI recombination target site results in deletion of the heterologous nucleotide sequence.

44. The method of claim 38 wherein the first TnpI recombination target site and the second TnpI recombination target site are in inverse orientation.

45. The method of claim 25, 26, or 35 wherein the cell contains a sequence encoding TnpI operably linked to a promoter.

46. The method of claim 45 wherein the promoter is an inducible or tissue-specific promoter.

47. The method of claim 25, 26, or 35 wherein the cell is a eukaryotic cell.

48. The method of claim 47 wherein the cell is a mouse cell.

49. The method of claim 47 wherein the cell is an embryonic stem cell.

50. The method of claim 22 or 35 wherein the first TnpI recombination target site and the second TnpI recombination target site are both TRT' sequences (SEQ ID NO:2) or functional variants thereof.

51. The method of claim 22 or 35 wherein the first TnpI recombination target site and the second TnpI recombination target site are both TRT sequences (SEQ ID NO:3) or functional variants thereof.

- 5 52. A method of producing a circular DNA vaccine comprising:
- a) introducing a DNA molecule into a non-*Bacillus thuringiensis* cell, said DNA molecule comprising, in the following order, from 5' to 3', a DNA sequence encoding an antigen of interest, a first TRT' site or functional variant thereof, an origin of replication and, optionally, one or more selectable markers, and a second TRT' site or functional variant thereof; and
- 10 b) contacting said cell with TnpI protein under sufficient conditions and in an amount sufficient to mediate site-specific recombination between the first and second TRT' sites or functional variants thereof, such that recombination between the first and the second TRT' sites or functional variants thereof results in deletion of the origin of replication and the optional one or more selectable markers from the DNA molecule,
- 15 such that a circular DNA vaccine encoding an antigen of interest is produced.
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